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With reference to the Written Opinion dated 2 June 2004, I hereby file a new set of claims, two (2) copies of publications and the following argumentation.

Claim 1 of the new claim set has been amended to a use claim directed to the use of a marker comprising an integrin alpha 10 chain or integrin alpha 10 and integrin alpha 11 chain expressed on the cell surface of a mesenchymal stem cell or intracellular in a mesenchymal stem cell as a marker for mammalian mesenchymal stem cells.

Thus, the application no longer comprises of the use of integrin alpha 11 only.

Claim 2 refers to the new main claim. Consequently, claims 1 and 2 are no longer directed to alpha 10 and /or alpha 11 integrin as such.

Claim 7 has been limited to mesenchymal stem cells that express integrin alpha 10 or integrin alpha 10 and integrin alpha 11.

Further, step b) of the new claim 9 has been limited to the use of a compound identifying

integrin alpha 10 chain or integrin alpha 10 and integrin alpha 11 chain expressed on the cell surface of a mesenchymal stem cell or intracellular in a mesenchymal stem cell.

The invention is based on the finding that integrin alpha 10 chain and/or integrin alpha 11 chain is/are expressed on the cell surface of a mesenchymal stem cell or intracellularly in a mesenchymal stem cell. Mesenchymal stem cells are pluripotent and undifferentiated and may be very useful for various purposes. Thus, there is a need for a marker for detecting and selecting them. None of the cited references show that these integrins may be found on or inside mesenchymal stem cells.

D1 shows that expression of alpha 10 occurs at the same time as chondrogenesis begins as judged by collagen type II expression – however, the paper provides no evidence that alpha 10 can be used to determine the onset of chondrogenesis, nor that expression of alpha 10 is responsible for the initiation of chondrogenesis. By definition chondrogenesis ('the development of cartilage') is a process whereby a committed mesenchymal stem cell passes through a number of cell phenotypes (see Fig 1 of the present PCT application) and whereby the cells continually change their repertoire of characteristic cell-surface markers to become a chondrocyte.

It should also be noted that during chondrogenesis, mesenchymal cells condense (not mesenchymal stem cells) to finally differentiate to chondrocytes. By inference, it is not known from D1 which cell type that alpha 10 is expressed upon during this process. Moreover, the method used in D1 (immunohistochemical staining) is a staining method for the detection of the expression of the integrin alpha 10. It does not enable identification a particular cell type, but merely shows that there are cells expressing the protein within any given tissue.

In conclusion, mesenchymal stem cells possess such pluripotentiality, that one can not assume from D1 that alpha 10 -expressing cells, which appear at the same time as the process of chondrogenesis occurs and cartilage is formed, are inherently those cells that are involved in chondrogenesis and therefore mesenchymal stem cells.

Moreover, the cells in the ossification groove cannot be defined as undifferentiated, mesenchymal stem cells since by reference (Shapiro F, Holtrop ME, Glimcher MJ. (1977)

J. Bone Joint Surg. Am. 59(6):703-23) they consist of: 1. a group of densely packed cells which are progenitor cells for the osteoblasts; 2. a group of more widely dispersed, **relatively** undifferentiated mesenchymal cells and fibroblasts, some of which are chondroblast precursors that **probably** contribute to appositional chondrogenesis and growth in width of the epiphyseal cartilage. 3. Fibroblasts and fibrocytes. This is supported by Morris NP et al (Connective Tissue and its Heritable Disorders 41-65 (2001) Ed: Royce PM and Steinman B, Pub: Wiley-Liss, Inc) who also describe the cells of the ossification groove of ranvier as being mesenchymal precursor cells (page 53 para2) and not mesenchymal stem cells or undifferentiated mesenchymal stem cells.

D2

This document regards mesenchymal nonmuscle cells. Like D1 it does not mention mesenchymal stem cells.

Even though D2 on p2 p117 of D mentions alpha11 in connection with mesenchymal nonmuscle cells it does not mention mesenchymal stem cells but cells derived from mesenchymal stem cells and therefore of mesenchymal origin. Rather, the document refers to areas of highly organized interstitial collagen networks p 117 right column lines 9-12 whish indicates that the cells are differentiated.

D3

Since the claims have been limited to integrin alpha 10 and integrin alpha 10 in combination with integrin alpha 11 this document is no longer citable since it refers to integrin alpha 11.

D4

Claim 9 has been limited to the identification and isolation of mesenchymal stem cells expressing integrin alpha 10 or integrin alpha 10 and an integrin alpha 11. Thus, this

document is no longer citable.

Stockholm, 26 July 2004

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Encls: New claims

2 Publications

CLAIMS

1. Use of a marker comprising an integrin alpha 10 chain or an integrin alpha 10 chain and an integrin alpha 11 chain expressed on the cell surface of a mesenchymal stem cell or intracellular in a mesenchymal stem cell as a marker for mammalian mesenchymal stem cells.

2. Use according to claim 1, wherein the integrin alpha 10 chain or the integrin alpha 10 chain and the integrin alpha 11 chain is/are expressed as a heterodimer(s) in combination with an integrin beta1 chain.

3. A method for identifying a mammalian mesenchymal stem cell, the method comprising the steps of
a) providing a sample comprising a mesenchymal stem cell,
b) detecting integrin chain alpha10 or integrin chain alpha10 and alpha11 expression on the cell surface of a mesenchymal stem cell or intracellular in a mesenchymal stem cell,
c) scoring the integrin chain alpha10 or integrin chain alpha10 and alpha11 expression, and
d) identifying the mesenchymal stem cell according to the scoring in c) above.

4. The method according to claim 3, wherein the expression in b) above is detected by detecting the integrin chain alpha10 or integrin chain alpha10 and alpha 11 protein expression.

5. The method according to claim 3, wherein the expression in b) above is detected by detecting the integrin alpha10 or the integrin alpha 10 and integrin alpha 11 mRNA expression.

6. The method according to any of claims 3-4, wherein the expression in b) above is detected by an immunoassay.

7. A method for determining whether a test compound modulates a mammalian mesenchymal stem cell differentiation, the method comprising the steps of
a) providing a mesenchymal stem cell that expresses integrin alpha10 or the integrin alpha 10 and integrin alpha 11
b) contacting the mesenchymal stem cell with a test compound, and

c) detecting a change in rate or pattern of differentiation of the mesenchymal stem cell as an indication of that the test compound modulates a mesenchymal stem cell differentiation.

8. The method according to claim 7, wherein the rate or pattern of differentiation is detected by detecting integrin chain alpha10 or integrin alpha 10 and integrin alpha11 expression on the cell surface of said mesenchymal stem cell or intracellular in a mesenchymal stem cell according to the method in any of claims 3-6.

9. A method for producing an isolated population of mammalian cells enriched for mesenchymal stem cells relative a reference population, the method comprising the steps of

a) providing at least a portion of a population of cells, or a portion of a reference population, comprising a mesenchymal stem cell and at least one cell other than a mesenchymal stem cells,

b) introducing into the population of cells in a) above a compound identifying an integrin alpha 10 chain or integrin alpha 10 and integrin alpha 11 chain expressed on the cell surface of a mesenchymal stem cell or intracellular in a mesenchymal stem cell,

c) selecting and isolating from the population of cells in b) above the mesenchymal stem cells, thereby producing a population of cells enriched for mesenchymal stem cells.

10. The method according to claim 9, wherein the mesenchymal stem cells is identified as a mesenchymal stem cell by detecting expression of integrin alpha10 or integrin alpha 10 and alpha11 chain expression on the cell surface of said mesenchymal stem cells according to the method in any of claims 3-6.

11. The method according to any of claims 9-10, wherein the selection in c) above is performed by fluorescent cell sorting.

12. An enriched mammalian cellular population of mesenchymal stem cells, comprising at least one intact, viable mesenchymal stem cell, wherein the mesenchymal stem cell are characterised by

a) expressing an integrin alpha 10 chain or integrin alpha 10 and integrin alpha 11 chain on the cell surface of or intracellular in said mesenchymal stem cell,

b) being substantially free from expression of molecules specific for committed

lymphohaematopoietic cells or uncommitted stem cells.

13. An isolated mammalian mesenchymal stem cell expressing a marker according to any of claims 1-2, obtainable by the method for producing a population of cells enriched for mesenchymal stem cells according to any of claims 9-10.

14. A mammalian cellular composition comprising the enriched cellular population according to claim 12, or the isolated mesenchymal stem cell according to claim 13.

15. Use of a marker according to any of claims 1-2, for identification of a mammalian mesenchymal stem cell.

16. Use of a marker according to any of claims 1-2, for modulating differentiation of a mammalian mesenchymal stem cell.

17. Use of a marker according to any of claims 1-2, for isolating a mammalian mesenchymal stem cell.